



BCH 447

Estimation of Serum Urea

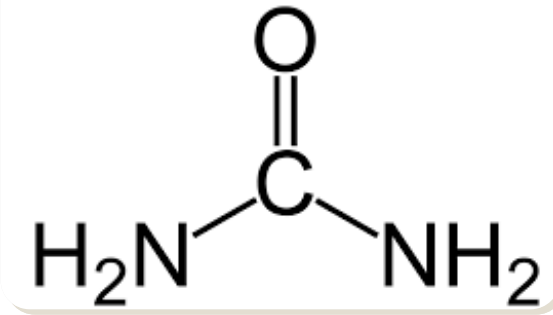
Objective:

- Estimation of Blood urea nitrogen (BUN) in serum sample.



-Urea:

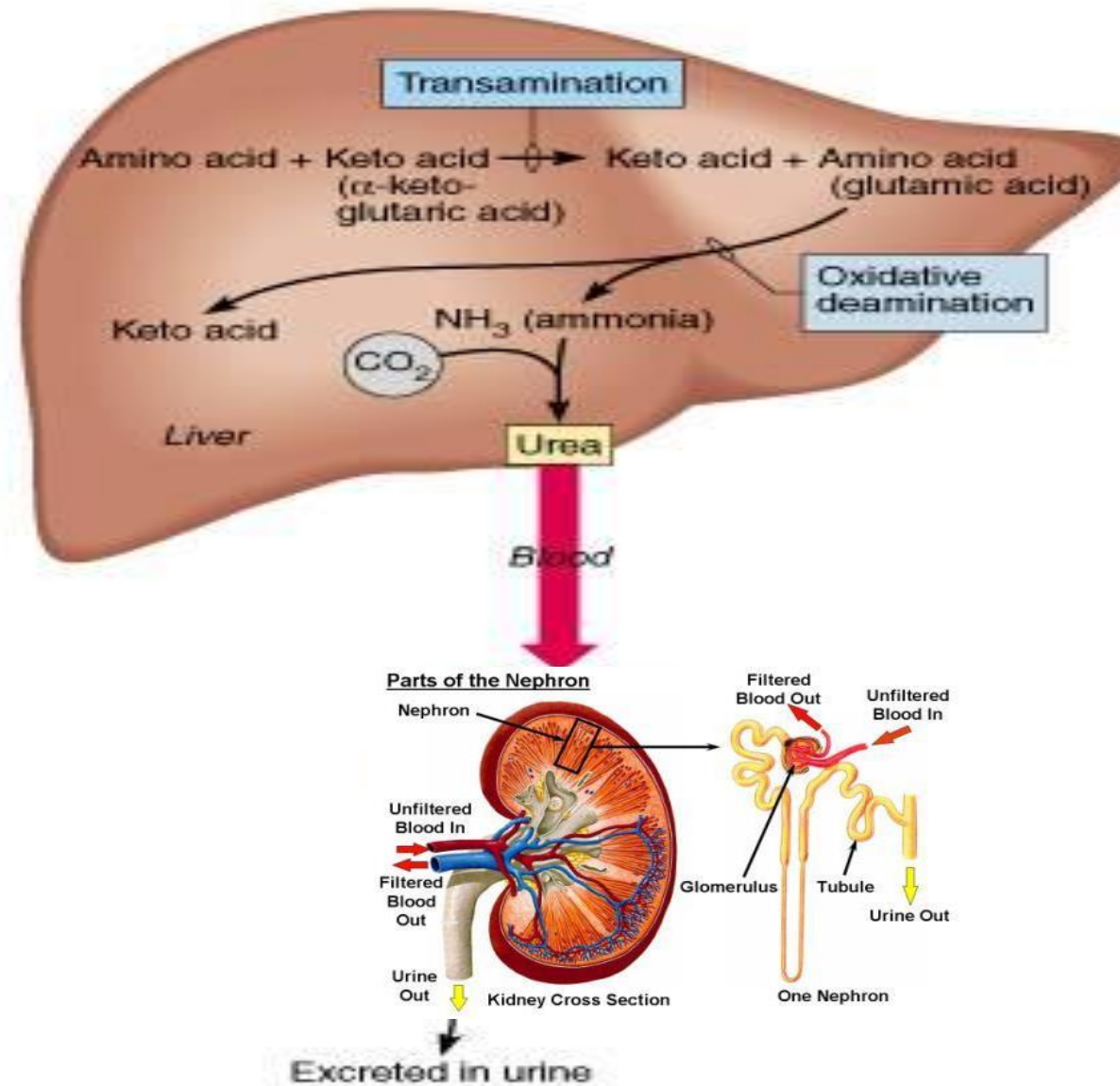
- Urea is the highest non-protein nitrogen compound in the blood.
- Urea is the major excretory product of protein metabolism.
- Since historic assays for urea were based on measurement of nitrogen, the term blood urea nitrogen (BUN) has been used to refer to urea determination.



-Urea synthesis:

- **Protein catabolism** produces amino acids that can be oxidized.
- This results in the release of **ammonia** which is **converted to urea** (via **urea cycle in the liver**).
- Following synthesis in the liver, urea is carried out in the **blood to the kidney** which is readily **filtered from the plasma by glomerulus**.
- **Most of the urea** in the glomerular filtrate excreted in the urine, and **some urea is reabsorbed** through the renal tubules.
- The amount reabsorbed **depends on urine flow rate and extent of hydration** (the amount of urea reabsorbed increases with dehydration).

-Urea synthesis:



- Clinical Application:

- **Measurement of urea used in :**
- Evaluate renal function.
- To assess hydration status.
- To determine nitrogen balance.
- To aid in the diagnosis of renal diseases.
- Check a person's protein balance.

-Plasma urea Concentration:

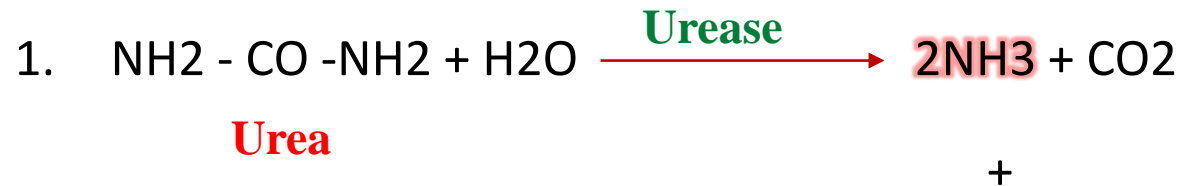
- Measurement of **Blood Urea Nitrogen (BUN)** alone is **less useful in diagnosing kidney diseases** because it's blood level is influenced by **dietary protein and hepatic function**.
- But its diagnostic value improves with **serum creatinine values**.

	Type	Cause	
High urea (High urea conc. in plasma is called azotemia)	Pre-renal	<ul style="list-style-type: none">• Cognitive heart failure. — —• Dehydration.• High protein diet.• Increased protein catabolism.	➔ Caused by reduced renal blood flow, less blood is delivered to kidney, then less urea is filtered.
	Renal	<ul style="list-style-type: none">• Renal failure .	
	Post-renal	<ul style="list-style-type: none">• Urinary tract obstruction.	
Low urea		<ul style="list-style-type: none">• Low protein intake.• Liver disease.• Pregnancy. — — — — ➔	➔ During pregnancy, The glomerular filtration rate increases by 50%

Practical Part

-Principle (of the kit used):

- **The Reagent used contains:** Urease, Glutamate Dehydrogenase (GLDH), NADH, α -ketoglutaric acid, buffers and stabilizers .
- **This test involves two reactions:**



- The absorbance at 340nm is measured over a limited time period, resulting in decreased readings due to the oxidation of NADH to NAD

-Method:

	Standard	Serum
Reconstituted Reagent	3ml	3ml
Pre-warm at 37°C for 2 min. and add:		
Standard	0.025/25μl	-
Serum	-	0.025/25μl

- After exactly 30 seconds . read and record absorbance A1 against distilled water at 340 nm.
- At exactly another 60 seconds after A1, read and record the absorbance A2 and determine ΔA (A1-A2).

-Calculations :

- Concentration of urea in serum sample:

- Standard concentration= 25 mg/dl

$$\text{- Urea (mg/dL)} = \frac{\Delta A (\text{Sample})}{\Delta A (\text{Standard})} \times 25$$

-Discussion:

- Comment on the level of Urea in serum .

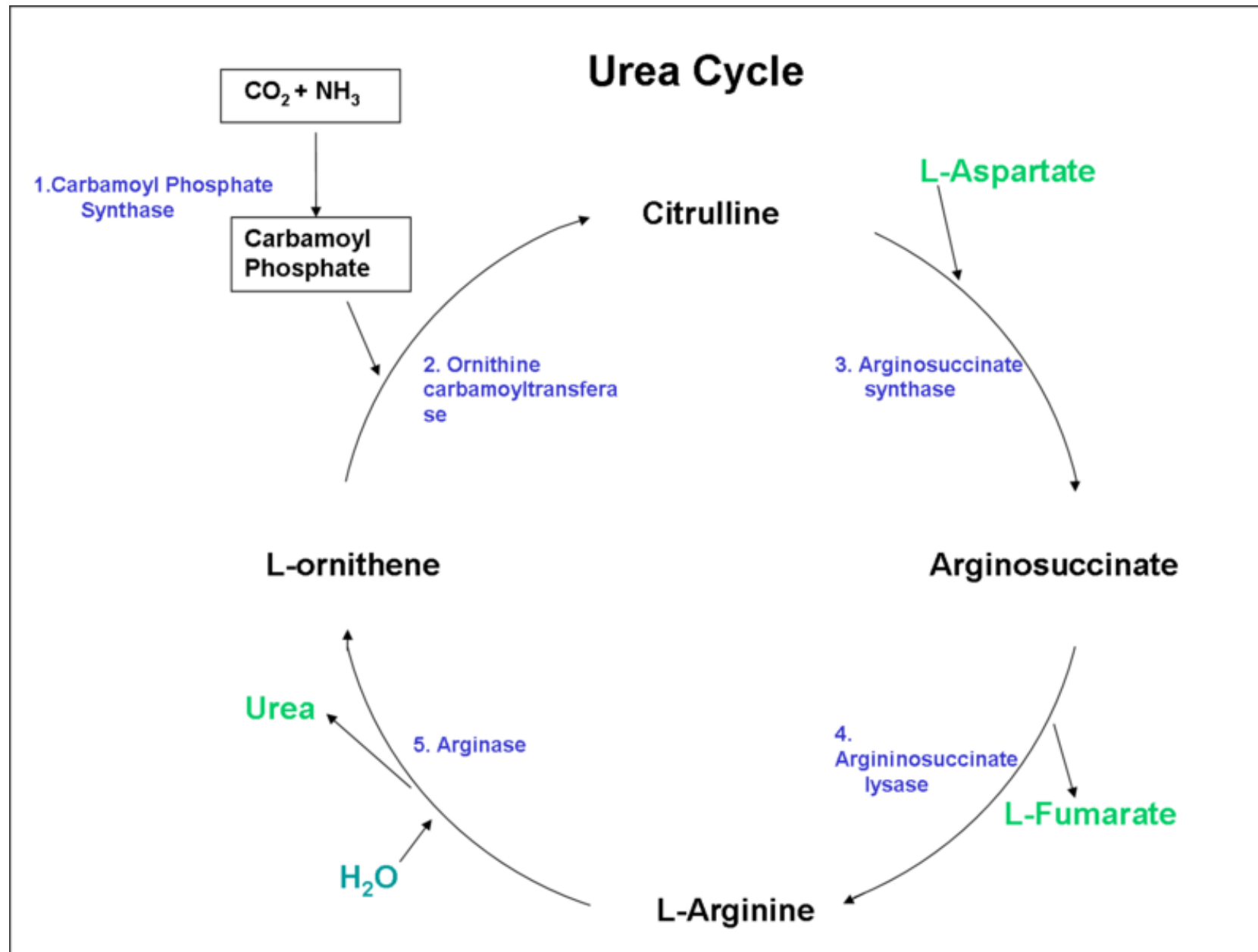
-Reference Value:

Blood urea conc.: (10 – 50 mg/ dl)

Estimation Of Arginase Activity In Liver Extract

- Introduction:

- Ammonia is a product of oxidative deamination of amino acids.
- It is toxic in even small amount and it must be removed from the body.
- Arginase is one of the important enzymes in urea cycle which is the major disposal form of amino groups derived from amino acids.
- Urea cycle catalyzed by a set of enzymes (Five enzymes) present in the liver ,and then is transported in the blood to the kidneys for excretion.



- Principle:

-The arginase enzyme catalyzes the fifth reaction in the urea cycle, the enzyme is present **exclusively in the liver** .

-Arginase catalyzes the hydrolytic cleavage of the guanidine group of Arginine to regenerate ornithine and urea.



-Two isozymes of this Enzyme exist ,

-First ; Arginase I (**In cytoplasm**) for functions of urea cycle,

- Second; Arginase II to regulate the arginine/ornithine concentration in the cell (**In mitochondria**).

- Arginase requires a two-molecules metal of **Co²⁺ and Mn²⁺** for it's activation while **ornithine and lysine** are potent inhibitors.

-The activity of the enzyme is determined by **measuring the amount of urea produced**, urea is reacted with the reagent iso-nitrosopropiophenone and heated in boiling water, leading to the production of a red color compound which is measured by spectrophotometry at 520nm.

Urea + iso-nitrosopropiophenone boiling water bath → **red color compound**

- Question:

- What are the causes of high blood ammonia level?**